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Review

Chemistry and Biology of the Clinically Used Macrolactone Antibiotic Fidaxomicin

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Fidaxomicin (**1**, lipiarmycin A3, clostomicin B1, tiacumicin B) constitutes a glycosylated 18-membered macrolactone and is a natural product isolated from various soil bacteria. Since 2011, fidaxomicin is a marketed antibiotic for the treatment of intestine infections caused by *C. difficile* in the clinic. Its promising *in vitro* antibacterial properties against resistant *S. aureus* and *M. tuberculosis* continue to attract interest. This review article describes the early history of the antibiotic fidaxomicin and highlights recent advances in the field, such as the elucidation of its mode of action and biosynthesis, as well as known derivatives. Furthermore, different synthetic strategies towards the total synthesis of fidaxomicin are summarized.

Keywords: Antibiotics • Biosynthesis • Fidaxomicin • Natural Product • Total Synthesis

1. Isolation, Structure Elucidation and Biological Activity

Fidaxomicin (**1**) is a natural product possessing an 18-membered, highly unsaturated macrolactone and is assigned as the first member of a new class of antibiotics.[1–3] Antibiotic **1** was first isolated in 1972 from an actinobacterium, *Actinoplanes deccanensis*, found in a soil sample from India.[4–6] The producer strain was isolated on February 29th, 1972 – in a leap year – and accordingly, the produced compounds were named lipiarmycins. Preliminary structural studies on lipiarmycin identified the rhamnoside-dichlorohomoorsellinate moiety as well as the modified noviose as important structural features (*Figure 1*).[7] However, lipiarmycin was later reported to actually compose of two compounds, lipiarmycin A3 (**1**) and A4 (**9**), whose structures were finally elucidated after extensive NMR and degradation studies.[8] Lipiarmycin A3 consists of an 18-membered macrolactone, which is larger than in common macrolide antibiotics (12- to 16-membered rings).[9,10]

Andrea Dorst received her Master's degree in 2015 from the University of Basel where she already joined the group of Prof. Dr. Karl Gademann to conduct her Master thesis on natural product synthesis. She then continued in the Gademann group that had just moved to the University of Zurich to perform her PhD studies on the synthesis of semisynthetic fidaxomicin analogs.

Karl Gademann (1972) currently serves as full professor and head of the Department of Chemistry at the University of Zurich, Switzerland. His previous professional affiliations include the University of Basel, EPFL Lausanne, ETH Zurich, and Harvard University, where he worked with Professors Seebach, Carreira (ETH). and Jacobsen (Harvard). His research interests include the biogenesis and chemical synthesis of natural products, and understanding of their function related to human endeavours.

The aglycon is at the C(11)-position connected to a modified D-noviose, which possesses an isobutyl ester instead of a methoxy group at C(4'')-position.In the C(20)-position the rhamnoside-dichlorohomoorsellinate moiety is attached.

In contrast, lipiarmycin A4 (**9**) was found to have a methyl group at the homoorsellinic acid in place of an ethyl group. However, the absolute configuration on the macrolactone was not yet assigned. Furthermore, lipiarmycins B3 (**2**) and B4 (**8**) were found and distinguished themselves from A3 (**1**) and A4 (**9**) by the position of the isobutyric ester.[11] While the isobutyric ester in **1** and **9** is attached at the C(4'')-hydroxy group, compounds **2** and **8** bear the isobutyric ester at the C(2'')-hydroxy group.

More than one decade after the first isolation, in 1986 a Japanese research group reported five related antibiotics from *Micromonospora echinospora* subsp. *armenica* subsp. nov., clostomicins A, B1, B2, C and D. [12,13] Clostomicin B1 was found to be identical with lipiarmycin A3 (**1**). Furthermore, natural product **1** was isolated a third time from *Dactylosporangium aurantiacum* subsp. *hamdenensis* subsp. nov. together with five additional analogs (tiacumicin A-F)[14,15] and later further new tiacumicin analogs were found that are only present in the culture broth in small quantities (compounds **5**-**7** and **15**-**17**).[16] In 2008, researchers from Novartis isolated macrolactone **1** again from *Catellatospora* sp. Bp3323-81.[17]

The configuration of the stereogenic center at C(18) was under discussion for a long time. In 2005, scientists from Optimer Pharmaceuticals enclosed the crystal structure of tiacumicin B in a patent and thereby unambiguously assigned the (*R*)-configuration for tiacumicin B and claimed an (*S*)-configuration for lipiarmycins, however, without supporting evidence.[18] In 2015, Serra and coworkers closer investigated lipiarmycin A3 and tiacumicin B by comparison of NMR spectra of degradation products and could provide evidence for the co-identity of those two natural products.[19] Furthermore, in 2017, the co-identity of lipiarmycin A3 and tiacumicin B was unambiguously verified by single crystal X-ray analysis and revealed the (*R*)-configuration, and therefore structural identity, for both lipiarmycin A3 and tiacumicin B.[20]

Fidaxomicin (**1**) was reported to be active against mainly Gram-positive bacteria including strains insusceptible to other commercial antibiotics. Although an anti-cancer activity was reported, investigations in these directions have been rarely reported.[21]

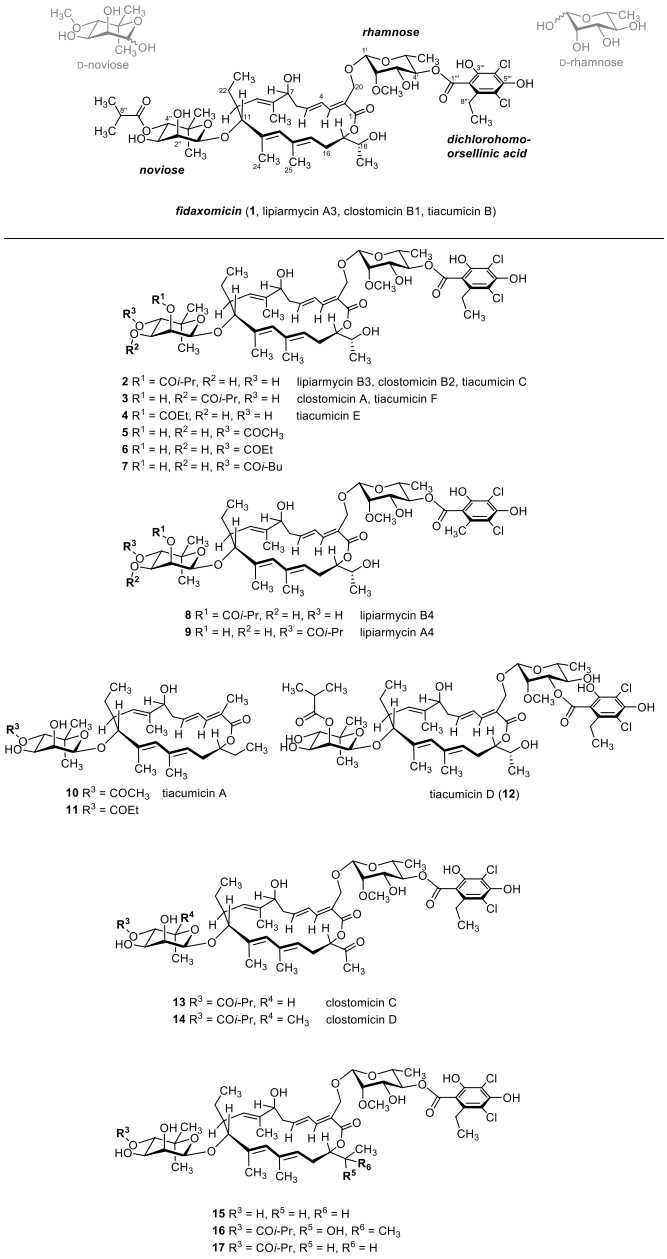


Figure 1. Structure of fidaxomicin (**1**) and naturally occurring analogs **2**-**17**.

Excellent activities were reported against *Clostridium difficile* (recently reclassified as *Clostridioides difficile*)[22] and *Clostridium perfringens* as well as resistant strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis*.[12,17,23,24] Cross-resistance was not observed with a broad panel of different antibiotics.[5,25]

Moreover, a strong pH dependence of the antibacterial properties was discovered. While compound **1** features good antibiotic activities at pH = 6, the minimum inhibitory concentration (MIC) increases around 100 times at pH = 8.6.[5] Incubation of **1** at different pH values and MIC determination at a fixed pH value did not result in any effect on the activity. The reversibility of the activity implies that pH dependence is due to the variation of permeation properties rather than degradation. However, a later report from Abbott Laboratories did not confirm this pH dependence in their tests.[23]

Lipiarmycin B (isobutyric ester in C(2′′)-position) is reported to be less active than antibiotic **1**.[11] Furthermore, all other isolated tiacumicin analogs were shown to be less active than the parent compound **1** against a panel of more than 20 different strains, including *Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus luteus*, *Enterococcus faecium* and others.[14] However, the antibacterial activity of tiacumicin F (**3**, isobutyric ester at C(3′′)-hydroxy group) is similar to **1**.

2. Clinical Application and Future Prospects

Although possessing activity against a variety of different bacterial strains, including some highly pathogenic bacteria that already developed resistance against some commercially available antibiotics, the clinical use of fidaxomicin (**1**) is limited to the treatment of *C. difficile* infections (CDI). In 2011, antibiotic **1** was approved for the treatment of CDI and it was marketed as Dificlir®. Treatments of infections outside the intestinal tract are not yet achieved, due to the low water solubility and thus low systemic absorption.[26,27]

The incidences of CDI are steadily increasing. In the latest report of the Centers for Disease Control and Prevention (CDC) in the U.S., an alarming trend was mentioned. In 2017, 223′900 people in the U.S. were infected with *C. difficile*, whereof 12′800 died. Therefore, *C. difficile* has been classified as a "urgent threat".[28]

CDI often occurs after treatment with antibiotics due to a damaged gut flora.[29] In this context, narrow spectrum antibiotics are of great interest, as they can combat pathogenic bacteria but are not harmful towards others. In this perspective, the narrow spectrum of antibacterial activity of **1** is advantageous. Indeed, it belongs to the most efficient antibiotics for the treatment of *C. difficile* infections to date. It is reported to show an improved clinical profile compared to vancomycin and metronidazole based on better cure and recurrence rates. Moreover, antibiotic **1** exhibits a 10- to 100-fold lower MIC values for *C. difficile* compared to other intestinal bacteria and therefore a harmful influence on the gut flora is reduced.[30] However, besides the hospital acquired CDI, more and more cases of community-acquired (individuals considered low risk to get infected) CDIs have been reported, which is associated with spread of more virulent strains.

A commonly used method to characterize different *C. difficile* strains is PCR ribotyping which is based on genetic similarity of different strains. Notably, in recent years the ribotypes (RT) evolved are hypervirulent, such as RT 027, which is in addition highly resistant to fluoroquinolones and other antibiotics.[31] No reports have been found on fidaxomicin resistant *C. difficile*, however, this might only be a matter of time.[32] Occurrence of the different RTs widely differs between geographical regions and changes over time.[33]

Besides its excellent antibacterial activity against *C. difficile*, macrolactone **1** also shows strong activity against resistant strains of *S. aureus* and *M. tuberculosis*, the pathogen causing tuberculosis (TB). Worldwide, TB is among the top ten causes of death and the World Health Organization (WHO) estimated that around 10 million people got infected in 2018 and around 1.5 million people died of it.[34] Even though, TB is treatable, the treatment can be considered tedious. A common therapy for the treatment of drug-susceptible TB disease envisages a long-term application (six months) of at least four different antibiotics to minimize development of resistances. Unfortunately, resistances are developed quite fast and spread globally.

Although not possessing ideal bioavailability, fidaxomicin's potential of becoming the next generation antibiotic for the treatment of a broad spectrum of infections is not yet exploited. In this perspective, fidaxomicin could be a promising starting point for the development of new antibiotics.

3. Derivatives by Fermentation Studies and Semisynthesis

Although being an interesting target for further modification, only few examples on semisynthetic modifications of fidaxomicin (**1**) are documented. In 1995, Abbott Laboratories filed a patent in which they reported on the synthesis of dimethylfidaxomicin **18** (*Figure 2*).[35] Methylation of the phenolic hydroxy groups was achieved using diazomethane. Although significantly losing activity against most investigated bacterial strains, an improved activity against *M. luteus* was reported. Furthermore, benzyl derivative **19** has been reported to show diminished RNAP-inhibitory activity.[36]

Besides these two particular examples, the first and only study on semisynthetic derivatives that yielded a greater variety of analogs was performed by researchers from Echem Hightech Co.[21] They synthesized diversely substituted benzylidene acetals on the diol of the noviose moiety (compounds **20**-**27**) and reported on their promising anti-cancer activities. However, no antibacterial activities have been mentioned.

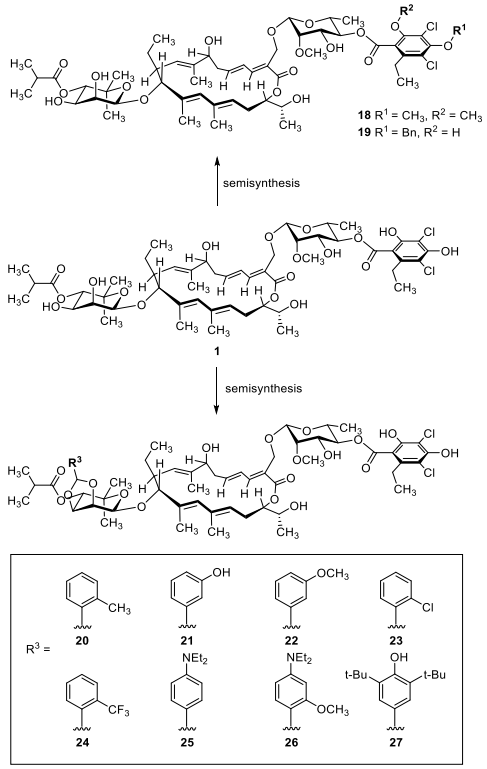


Figure 2. Structures of known semisynthetic derivatives of **1**.

Moreover, further novel fidaxomicin analogs were produced by fermentation (*Figure 3*). Feeding experiments revealed that chloride sources were essential for the successful production of **1**. By replacing all chloride sources by bromide, four new brominated fidaxomicin compounds have been isolated.[37,38] Based on antibacterial susceptibility tests, compounds **28** and **30** are more active against *C. difficile* and *C. perfringens*, while less active against *S. aureus*, *S. epidermis* and *E. faecium*.

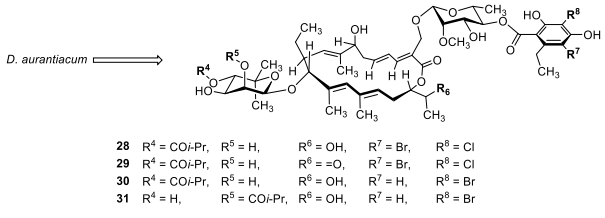


Figure 3. Known derivatives of **1** obtained by feeding experiments.

4. Investigation of the Biosynthetic Pathway

Four decades after the discovery of fidaxomicin (**1**), the group of Zhang and coworkers first reported on the biosynthetic pathway by the characterization of the biosynthetic gene cluster of *D. aurantiacum* subsp. *hamdenensis*, one of the known producer strains.[39] According to sequence analysis and analysis of truncated products isolated from the fermentation broth of gene-knockout mutants a possible biosynthetic pathway was proposed.[40–42]

They found four genes (*tiaA1-A4*) encoding for a modular type I polyketide synthase (PKS) that is responsible for the synthesis of the aglycon **32** (*Figure 4*). The PKS consist of a loading module and eight extending modules. The first step of the biosynthesis is the loading of the acyl carrier protein (ACP) with the starter unit, which is proposed to be propionyl-CoA. This propionyl starter is then translocated onto a ketosynthase (KS) domain of module 1. Next, the extender unit malonyl-CoA (**33**) is loaded onto the ACP of module 1 catalyzed by an acyltransferase (AT) and the decarboxylative Claisen condensation between propionyl and malonyl units takes place to furnish the diketide moiety, which is subsequently reduced by a ketoreductase (KR). In this fashion, the whole linear chain is synthesized and modified by KR or dehydratase (DH), which installs the alkene moieties upon elimination of water. Finally, the linear chain is cyclized and released by a thioesterase (TE) to give the macrolactone **32**.

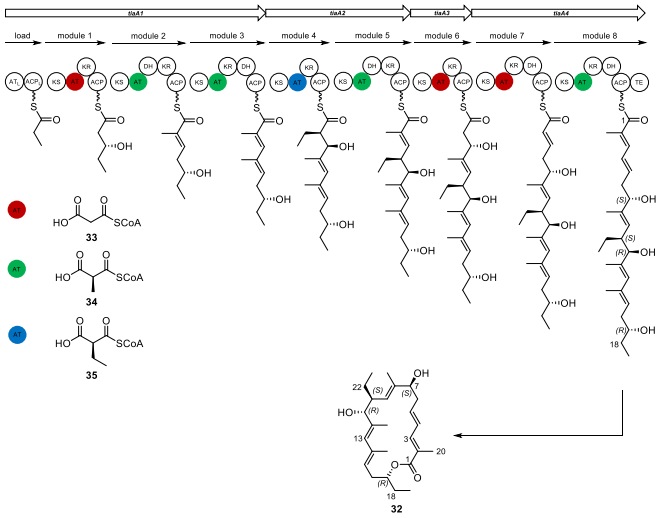
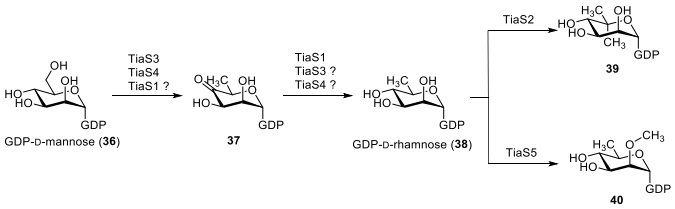


Figure 4. Proposed biosynthetic pathway towards fidaxomicin aglycon scaffold **32** by a multifunctional type I modular polyketide synthase (PKS). *tiaA1-A4*: genes encoding for modular polyketide synthases; AT: acyltransferase specific for malonyl-CoA (red), methylmalonyl-CoA (green), ethylmalonyl-CoA (blue); ACP: acyl carrier protein; KS: ketosynthase; KR: ketoreductase; DH: dehydratase; TE: thioesterase.[39]

The biosynthesis of the carbohydrate units is not yet fully understood. It is known that the noviose moiety **39** and rhamnose moiety **40** emerge from GDP-D-mannose (**36**) catalyzed by GDP-mannose-4,6-dehydratase (Gmd) and GDP-4-keto-6-deoxymannose reductase (Rmd) (*Scheme 1*).[43] In fact, three genes were found in the tiacumicin gene cluster that presumably encode for Gmds, namely *tiaS1*, *tiaS3*, *tiaS4*. However, no genes were found that are supposed to encode for Rmd, which catalyzes the reduction of ketone **37** to rhamnose **38**. Therefore, it was assumed, one of these enzymes (TiaS1, TiaS3 or TiaS4) could act as an Rmd as well. This assumption is supported by the existence of bifunctional Gmds that are able to catalyze such reductions.

From GDP-D-rhamnose (**38**) as a common intermediate, both carbohydrate moieties **39** and **40** are biosynthesized through methylations induced by *C*-methyltransferase TiaS2 and *O*-methyltransferase TiaS5, respectively. To confirm the role of TiaS2 in the biosynthesis, corresponding gene-knockout mutants were

prepared and an analog with a lacking methyl group on the C(5′′)-position of the noviose (compound **84**) was identified, besides some products lacking the noviose completely, for example **32** and **49** (for structures see below, *Figure 5*). A *tiaS5*-knockout mutant produced analogs, all lacking the C(7′)-methoxy group (major compounds **74** and **78**).



Scheme 1. Proposed pathway for the biosynthesis of the modified noviose **39** and rhamnose **40**. TiaS1, S3, S4: GDP-mannose-4,6-dehydratase, TiaS2: *C*-methyltransferase, TiaS5: *O*-methyltransferase.

The biosynthesis of the aromatic homoorsellinic acid moiety **47** is again derived from a type I PKS. Similar to already known orsellinic acid synthases, tiaB is proposed to use a propionyl-CoA (**45**) instead of an acetyl-CoA starter unit, resulting in the installation of an ethyl group in resorcinol **47** (*Scheme 2*). Therefore, using propionyl-CoA as starter unit and three malonyl-CoA as extender units the linear intermediate **46** is generated, which upon decarboxylative Claisen - condensation, dehydration and aromatization furnishes the homoorsellinic acid **47**.

For further elucidation of the final assembly of natural product **1**, Zhang and coworkers prepared and cultivated gene-knockout mutants of *D. aurantiacum* and analyzed the produced shunt product in order to get detailed information about this process.

Thereby, TiaP1 and TiaP2 were identified as cytochrome P450s and TiaG1 and TiaG2 as glycosyltransferases.

To probe the functions of these enzymes, the corresponding genes were inactivated. The *tiaG1*-mutants were found to produce compounds **32**, **41**, **48**, **49** and **50** lacking the noviose moiety and thereby confirming its role as glycosyltransferase for the noviose. In contrast, *tiaG2*-mutants produced compounds lacking the rhamnose resorcylate moiety (**42**, **51**-**53**) and thereby establishing the function as glycosyltransferases specific for rhamnosylations. Moreover, *tiaP1*-mutant failed to produce **1**, solely compound **17** with the missing C(18)-hydroxy group was isolated and *tiaP2*-mutant produced compounds lacking the C(20)-hydroxy group (compounds **10**, **11**, **54**-**56**).

The complete catalytic order of the assembly of the final natural product **1** remains elusive. However, latest investigations[42] indicated that TiaP2 first oxidizes macrocycle **32** at the C(20)-position, before glycosyltransferase TiaG1 attaches the noviose to form alcohol **42**. Then, the isobutyric ester is attached by acyltransferase TiaS6 or rhamnosylation occurs to construct fidaxomicin scaffold **43**. The homoorsellinic acid fragment **47** is then attached. In successive steps, the C(7′)-methyl group and the halogenation occur. The last step is proposed to be the oxidation of C(18)-position to furnish antibiotic **1**.

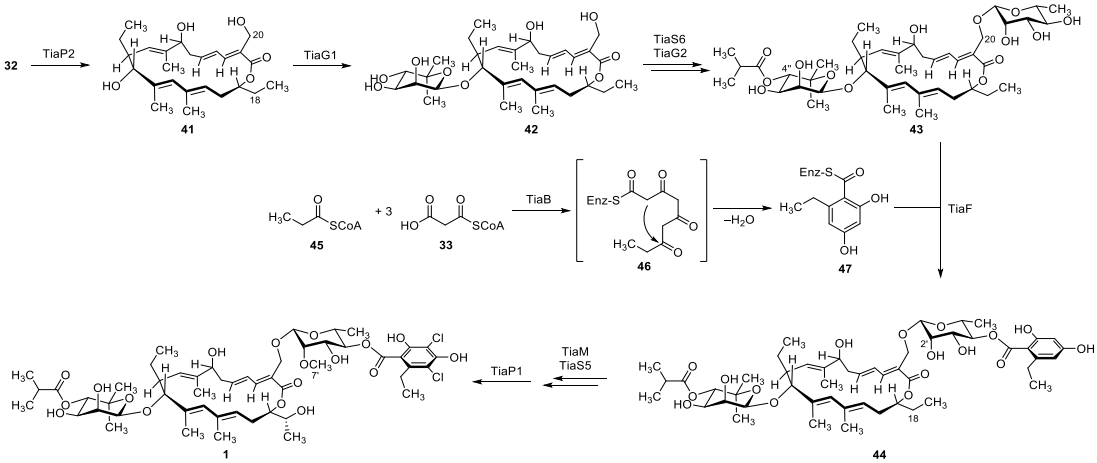
All fidaxomicin analogs obtained by Zhang and coworkers are summarized in *Figure 5* along with their reported antibacterial activities against *S. aureus*. For a more detailed summary, the interested reader is referred to reference 44.[44]

Although, antibacterial activity has not been reported for all compounds, a general trend is visible. Accordingly, the noviose and rhamnose sugar moiety are crucial for the antibacterial activity of **1** and analogs missing both or one of these moieties completely lose their antibacterial properties with the exception of denoviosylated **49** and **50**, which maintain some antibacterial activity even though significantly decreased compared to the parent compound **1**.

Notably, a propyl (analog **70**) instead of an ethyl (analog **68**) or methyl group (analog **69**) on the homoorsellinic acid with lacking chloride substituents increase the antibacterial activity.

Ambiguous results are observed for compounds lacking the C(18)-hydroxy group. Interestingly, compounds lacking the C(18)-hydroxy group and C(7′)-methyl group with different ester moieties attached to the noviose (**78**, **88**, **92**) retain/improve their activities, while compound **76** with the C(18)-hydroxy group attached and missing C(7′)-methyl displays decreased activity.

Oxidation in the C(18)-position to ketone **81** is not tolerated and the activity is diminished, whereas methylation in this position, as in compound **82**, displays improved activity compared to **1**. All in all, structure-activity relationships are indistinct and do not seem to be additive. Moreover, direct comparison of the compounds is usually difficult due to simultaneous changes at several positions. Modification on one position does not necessarily lead to a loss in activity and is also dependent on the influences of other positions.



Scheme 2. Proposed biosynthetic pathway to homoorsellinic acid **47** and assembly of natural product **1**. TiaB: iterative type I polyketide synthase (PKS); TiaF: Ketoacyl-ACP synthase; TiaG1, G2: glycosyltransferases; TiaM: halogenase; TiaP1, P2: cytochrome P450; TiaS5: *O*-methyltransferase; TiaS6: *O*-acyltransferase.

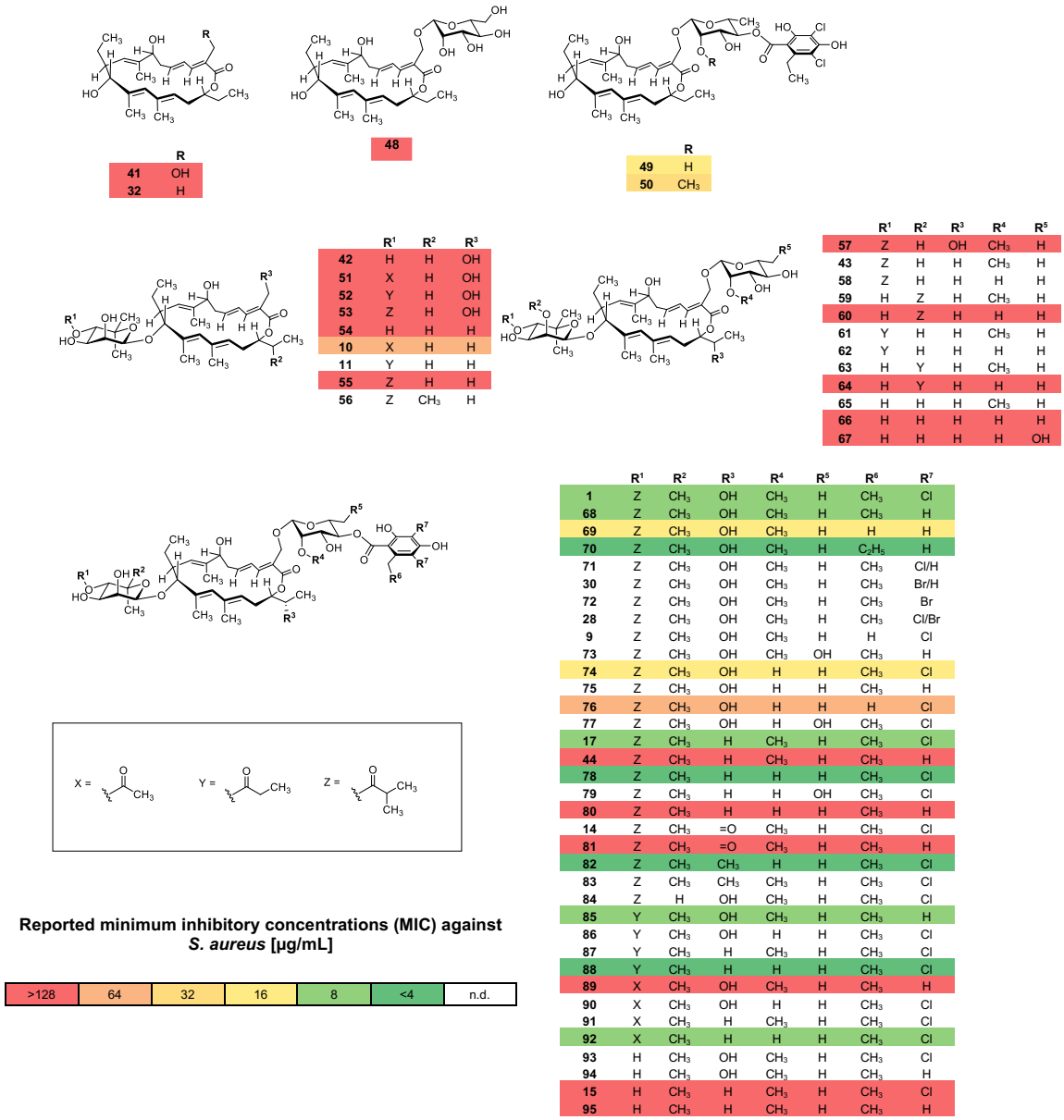


Figure 5. Reported fidaxomicin analogs obtained by fermentation of gene-knockout mutants and their reported antibacterial activities against *S. aureus*.

5. Mechanism of Action

The first report on the mechanism of action of fidaxomicin (**1**) appeared soon after the disclosure of the first isolation. Therein, Parenti and coworkers reported that antibiotic **1** interferes with the RNA synthesis by inhibiting RNA polymerase (RNAP).[45] The bacterial RNAP core enzyme consists of five subunits (ααββ′ω) and an initiation factor σ (*Figure 6*). Upon binding of the σ factor, the so-called holoenzyme is formed. Prokaryotic RNAP widely differs from eukaryotic RNAP and therefore renders this enzyme a convenient target for antibiotics as they specifically act on bacterial RNAP. During initiation of the transcription, the σ-factor needs to bind to the core enzyme in order to enable binding of the DNA-strand.[46–48] The promoter specific σ-factor consist of separate domains 1-4. The domains 2 and 4 recognize the −35 and −10 promoter sequences of the DNA, respectively, and forms a closed promoter complex (RP_C). Next, the double-stranded DNA is melted to form an open promoter complex (RP_O) onto which nucleotides can adhere to build up a new RNA strand.

Early on in the investigations on the mechanism of action, Parenti and coworkers found when fidaxomicin was added before initiation, the RNA synthesis was immediately discontinued, while addition of fidaxomicin at an advanced state, when nucleotide polymerization has already started, resulted in proceeding of RNA production for several minutes before it was suppressed.[45] This finding indicated that fidaxomicin interferes with the initiation step rather than elongation. Similar results were independently obtained by Clerici and coworkers.[49]

In subsequent years further progress was made towards the elucidation of the actual binding site. Studies on *B. subtilis* mutants revealed that fidaxomicin most likely binds to the β-subunit, but also the σ-factor was essential for inhibition by interacting with the holoenzyme and it was assumed that the formation of the first phosphodiester bond is blocked.[50,51] Furthermore, it was reported that besides the β-subunit also mutation in β′-subunit lead to resistance against fidaxomicin in *B. subtilis*[50,52] and *M. tuberculosis*.[17]

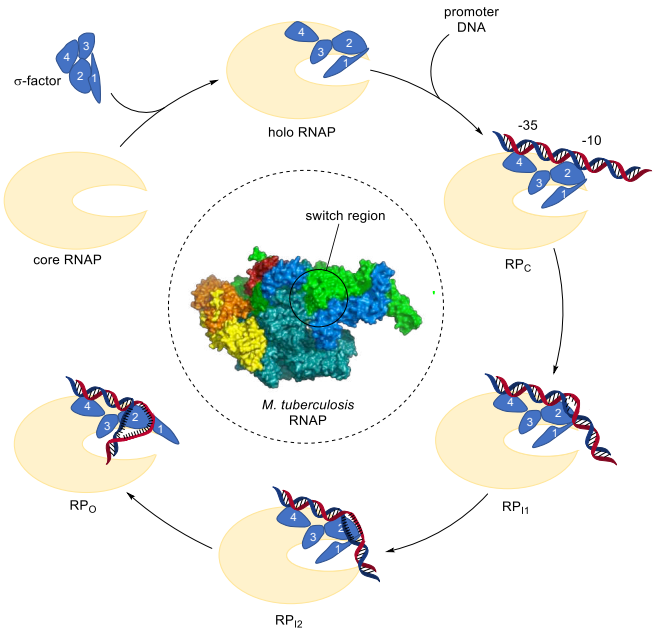


Figure 6. *M. tuberculosis* RNAP holo enzyme in molecular surface representation (PDB: 6FBV)[36] and transcription initiation pathway. Figure adapted from reference.[46]

Later, it was found that fidaxomicin most probably targets the switch region and/or the RNA-exit channel. In RNAP, the β- and β′-subunits form a pincer-like structure and the two subunits act as mobile clamps that can open or block the access to the active site by conformational changes. The switch-region is at the base of the RNA clamp and induces these necessary conformational changes. It was therefore proposed, that fidaxomicin traps one of the inactive and closed intermediates and prevents isomerization of RP_C to RP_O.[53–56] However, the role detailed role of the σ-subunit, which is required for promoter recognition and DNA unwinding remained elusive. Brodolin and coworkers studied the action of σ-subunit mutants and found that fidaxomicin competes with the −10 promoter sequence for the same binding site and thereby preventing the formation of the RP_O.[57]
The final breakthrough came with the elucidation of the cryo-electron microscopy (cryo-EM) structure of fidaxomicin binding to *M. tuberculosis* RNAP, independently disclosed in 2018 by the research groups of Campbell and Ebright, respectively.[36,58] These cryo-EM structures confirmed the interactions of **1** with the β, β′ as well as σ-subunits. Moreover, the cryo-EM structure as well as additional fluorescence resonance energy transfer (FRET) measurement indicated that fidaxomicin traps an open-clamp conformation through interaction with the switch-region and thereby traps a wrong spatial orientation of σ2- and σ4-units required for simultaneous recognition of −10 and −35 promoter elements.
To summarize, fidaxomicin constitutes an RNAP inhibitor. It inhibits transcription of DNA into RNA by binding to the switch-region and thereby trapping an open clamp conformation. This prevents simultaneous binding to the −35 and −10 promoter elements and promoter melting and thus formation of the transcription bubble (RP_O) is not possible anymore.
Besides fidaxomicin another prominent member of RNAP inhibitors, rifampicin, made it to the market. However, a different mode of action accounts for its antibacterial activity. Instead of interfering with the initiation, rifampicin binds to the β-subunit close to the RNA channel and thereby sterically prevents elongation of the newly formed RNA strand.[59]
Further mode of actions of antibiotics known to inhibit RNAP (not marketed drugs) include disruption of holoenzyme assembly, blocking conformational dynamics required for nucleotide addition and blocking nucleoside triphosphate (NTP) uptake.[48]

6. Total Syntheses

Due to its interesting antibacterial properties and chemical scaffold, fidaxomicin (**1**) attracted the interest of several research groups aiming for the total synthesis of this complex natural product. In late 2014, besides our group[60], also the research groups of Zhu[61] and Altmann[62] independently accomplished a synthesis towards the protected aglycon which were published at the same time. While Gademann and Altmann aimed for the actual (*R*)-configuration at C(18), Zhu synthesized the (*S*)-isomer (*Figure 7*). At that time, the co-identity of tiacumicin B and lipiarmycin A3 has not yet been demonstrated. Later in 2017, the group of Roulland and coworkers in France also achieved the synthesis of the protected aglycon.[63–65]

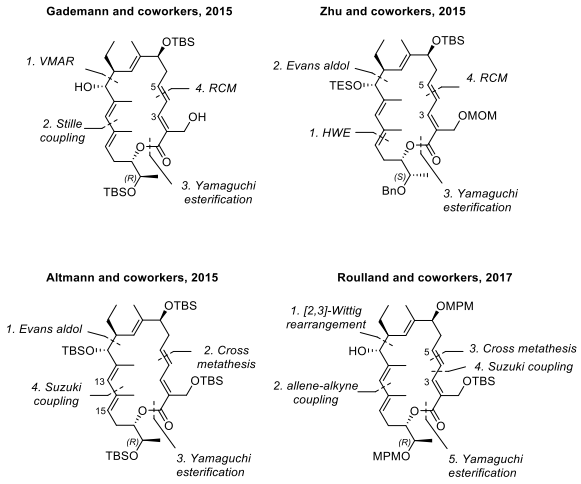
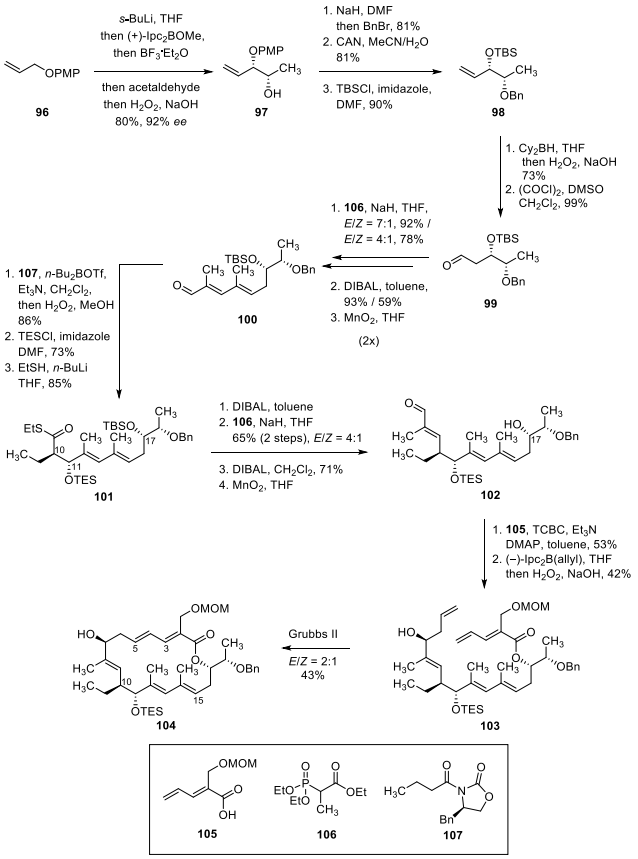


Figure 7. Summary of the different synthetic approaches towards the synthesis of the fidaxomicin aglycon.

Although the synthetic strategies resemble each other, the order of assembly of the fragments vary between the different approaches. For instance, Gademann and Zhu applied a ring closing metathesis (RCM) between C(4) and C(5) for the final ring closure, while Altmann chose a Suzuki cross coupling between C(13) and C(14) and Roulland engaged a Yamaguchi macrolactonization.
Finally, in 2015 our group accomplished the assembly of natural product **1** and published the first total synthesis thereof.[66] A further total synthesis of another member of the fidaxomicin family, tiacumicin A (**10**), followed in 2018 by our group.[67] Recently, Roulland and coworkers also achieved their total synthesis of **1** using optimized glycosyl-donors for the selective β-glycosylation.[68] In the following section, the different approaches will be presented and compared. Apart from these syntheses, also total syntheses of natural products containing a similar macrocyclic scaffold, mangrolide A and D, have been recently synthetically prepared.[69–71]

Zhu's Aglycon Synthesis

Zhu and coworkers[61] started their synthesis with a Brown allylation of acetaldehyde with protected allyl alcohol **96** to afford *syn*-diol **97** in 92% *ee* (*Scheme 3*). After a protection and deprotection sequence, the obtained alkene **98** was submitted to hydroboration/oxidation to afford the corresponding alcohol followed by a Swern oxidation to give aldehyde **99**. Next, the methyl alkene moiety was installed using a Horner-Wadsworth-Emmons (HWE) reaction with phosphonate **106** and subsequent reduction and oxidation sequence furnished an unsaturated aldehyde which was again submitted to the same reaction sequence to afford the aldehyde **100** with both methyl alkene moieties installed. Aldehyde **100** was then successfully engaged in an Evans aldol reaction with chiral enolate of **107** to



Scheme 3. Total synthesis of the protected fidaxomicin aglycon **104** by Zhu and coworkers. CAN: cerium ammonium nitrate; PMP: *p*-methoxyphenyl; Ipc: *i*-pinocampheyl; DIBAL: diisobutylaluminium hydride; TCBC: 2,4,6-trichlorobenzoyl chloride; DMAP: 4-dimethylaminopyridine.

install the neighboring stereocenters at C(10) and C(11). The generated hydroxy group was protected as a TES ether and the thioester **101** was installed using lithium ethyl thiolate. Subsequent reduction of thioester **101** to the corresponding aldehyde with DIBAL also led to the chemoselective removal of C(17)-TBS group. Another HWE reaction with phosphonate **106** furnished, upon reduction and oxidation, the aldehyde **102**. Next, a Yamaguchi esterification was applied to attach fragment **105**. Subsequently, allylation of the aldehyde function with (−)-Ipc$_2$B(allyl) then afforded the desired diastereomer **103**. As a final step, Zhu and coworkers performed a ring closing metathesis (RCM) using Grubbs' second-generation catalyst to afford the desired (*E*)-macrolactone **104** together with the undesired (*Z*)-isomer as a 2:1 mixture in 22 steps and 0.5% overall yield.

Altmann's Aglycon Synthesis

In contrast to the linear synthetic approach of Zhu and coworkers, the group of Altmann chose a rather convergent approach.[62] Thereby, they first envisioned the synthesis of fragment **111** and **118** which were later combined to assemble the desired macrolactone **121** (*Scheme 4*).

Starting from the known allylic alcohol **108**, oxidation to the corresponding aldehyde and subsequent Evans aldol reaction with the boron enolate of **112** furnished the *syn*-aldol product in high diastereoselectivity. Next, the oxazolidone was converted into the Weinreb amide **109**. TES protection of the secondary hydroxy group followed by DIBAL reduction of the Weinreb amide furnished the corresponding aldehyde in high yields. A Corey-Peterson olefination with imine **113** was applied to install the methyl alkene moiety and subsequent hydrolysis of the imine afforded aldehyde **110** in high *E/Z* selectivity. Finally, allylation of aldehyde **110** with Leighton's silacycle **114** and subsequent TBS protection of the hydroxy group produced the desired fragment **111**.

The synthesis of the second fragment **118** commenced with a Sharpless kinetic resolution of racemic allylic alcohol **115** followed by epoxide opening with *in situ* generated propynyl lithium to afford alkyne **117**. Finally, a copper-catalyzed borylation was applied to give the boronate **118**.

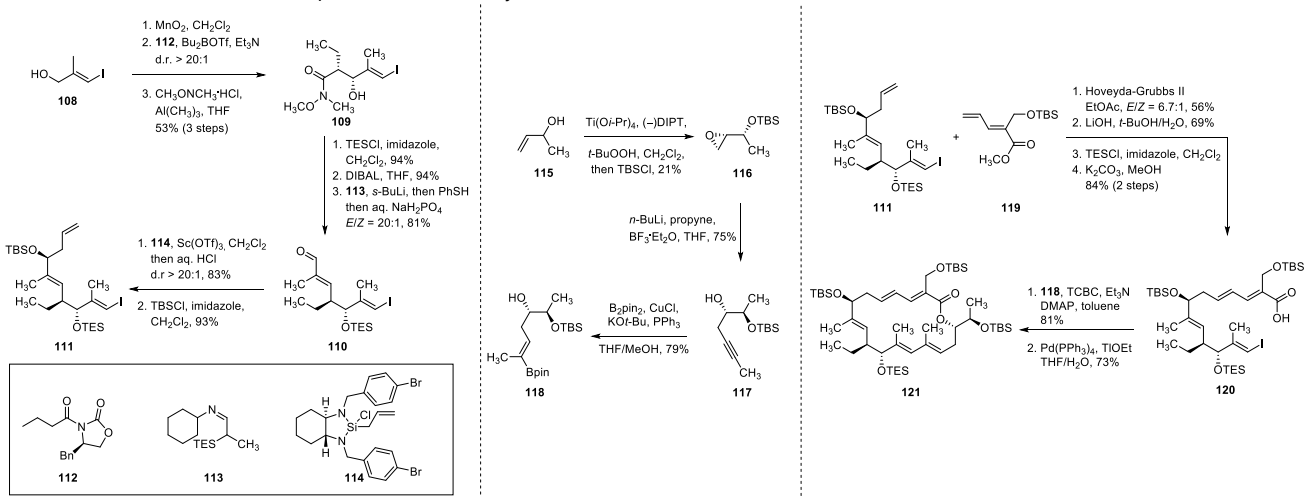
The assembly of the macrolactone then started with a cross metathesis using the second generation Hoveyda-Grubbs catalyst followed by hydrolysis of the methyl ester moiety. Due to unintentional TES deprotection the material was resubmitted to TES protection conditions to give carboxylic acid **120**. Next, a Yamaguchi esterification with fragment **118** and the final Suzuki cross coupling delivered the protected aglycon **121** in 14 steps (longest linear sequence) and an overall yield of 6%. Furthermore, global deprotection of the silyl groups to furnish unprotected aglycon and selective partial deprotection have been successfully conducted as well.

Gademann's Aglycon Synthesis

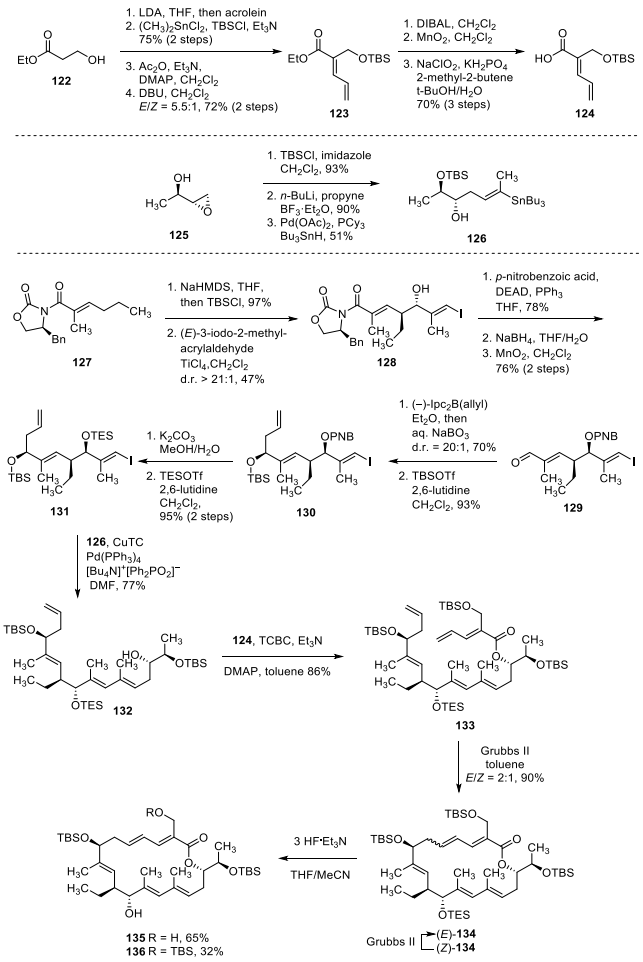
Similar to Zhu's approach, Gademann and coworkers envisioned the final ring closure *via* an RCM by assembly of three different fragments **124**, **126** and **131** (*Scheme 5*).[60]

The synthesis of fragment **124** was achieved starting from ester **122** *via* an aldol reaction with acrolein. Subsequently, the primary hydroxy group was selectively protected with TBSCl using a dimethyltin dichloride mediated protocol, followed by acetylation of the secondary hydroxy. DBU then induced acetate elimination to give dienoate **123**.

After the reduction of the ethyl ester **123** and oxidation to the aldehyde, a Lindgren-Pinnick oxidation furnished the desired carboxylic acid fragment **124**.



Scheme 4. Total synthesis of the fidaxomicin aglycon **121** by Altmann and coworkers. DIPT: diisopropyl D-tartrate; Bpin: pinacolatoboron.



Scheme 5. Total synthesis of the fidaxomicin aglycon **135** and **136** by Gademann and coworkers. LDA: lithium diisopropylamide; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DEAD: diethyl azodicarboxylate; HMDS: hexamethyldisilazane; PNB: *p*-nitrobenzyl.

The second fragment was synthesized in a three-step procedure starting from epoxide **125**. TBS protection of the hydroxy group was followed by epoxide opening with propynyl lithium in presence of Lewis acid $BF_3 \cdot Et_2O$. Finally, Pd-catalyzed hydrostannylation delivered the Stille coupling precursor **126** in moderate yields due to low regioselectivities.

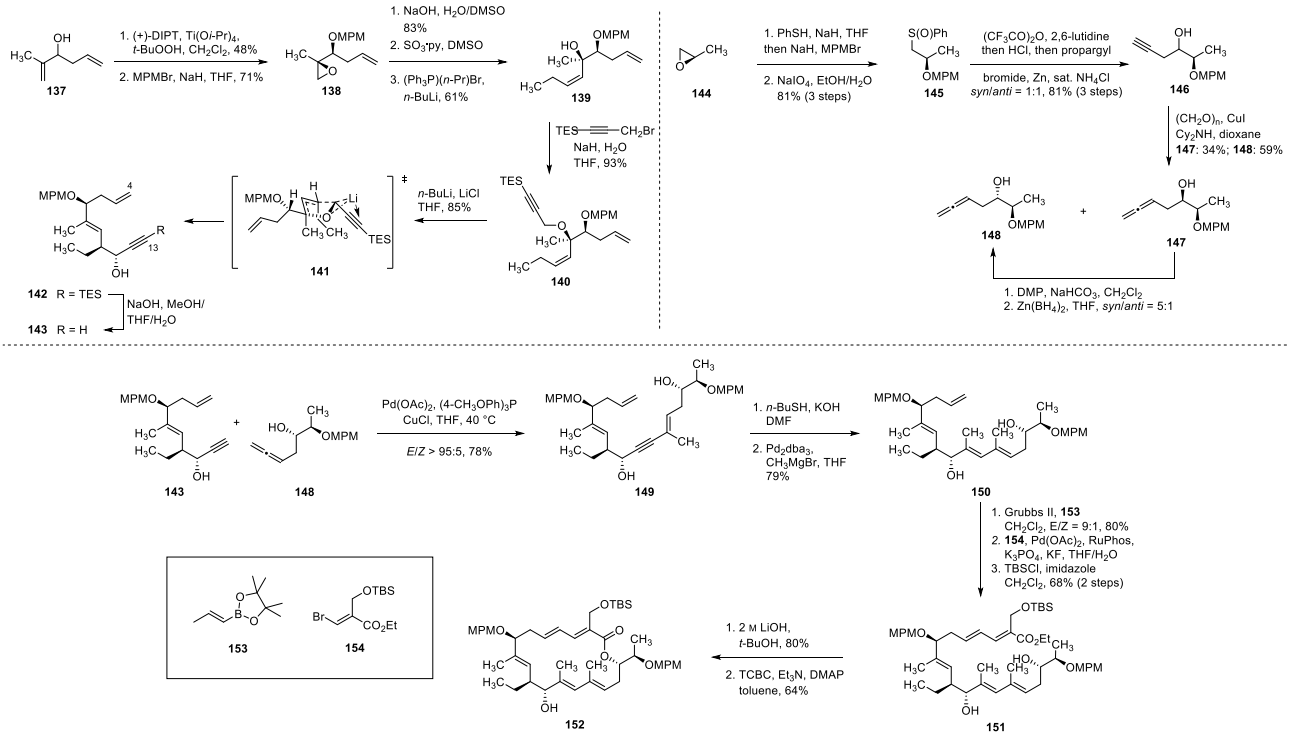
Having fragments **124** and **126** in hands, the final assembly was performed. Starting from known building block **127**, a silyl enol ether formation and subsequent vinylogous Mukaiyama aldol reaction (VMAR) with (*E*)-3-iodo-2-methylacrylaldehyde furnished iodide **128**. Alcohol **128** was submitted to PNB protection using Mitsunobu conditions with inversion of the stereocenter, followed by cleavage of the oxazilidinone auxiliary under reductive conditions. Next, MnO_2-mediated oxidation to aldehyde **129**, successive Brown allylation and TBS protection delivered silyl ether **130**.

Then, a protecting group exchange from PNB to TES ether **131** was required to successfully apply this substrate in a Stille cross coupling reaction to furnish **132** in good yields. Finally, Yamaguchi esterification with fragment **124** followed by RCM with second generation Grubbs catalyst delivered the desired protected aglycon **134** in an *E/Z* ratio of 2:1. Resubmission of the undesired (*Z*)-**134** to

the metathesis conditions induced isomerization and enabled recycling of the (*Z*)-isomer to afford more of the desired (*E*)-**134**. Additionally, TES and primary TBS deprotection delivered macrolactones **135** and **136** which could be directly engaged in glycosylation attempts towards the total synthesis of **1**.

Roulland's Aglycon Synthesis

The latest report of the aglycone synthesis was published by Roulland and coworkers using the C(4)-C(13) fragment **143** as key intermediate (*Scheme 6*).[63,64] For the synthesis of alkyne **143** a [2,3]-Wittig rearrangement was applied as a key step. Therefore, the precursor **140** was prepared starting from diene **137** using a Sharpless epoxidation, followed by protection as methoxybenzyl ether (MPM) **138**. Then, epoxide opening, Parikh-Doering oxidation and the subsequent Wittig olefination delivered alcohol **139**. After extensive screening, it was found that alkyne **140** smoothly undergoes Wittig rearrangement upon treatment with *n*-BuLi and LiCl in high diastereoselectivity to furnish, after TES deprotection, alkyne **143**.



Scheme 6. Total synthesis of the fidaxomicin aglycon **152** by Roulland and coworkers. MPM: *p*-methoxybenzyl; DMP: Dess-Martin periodinane.

Next, the synthesis of allene **148** commenced with epoxide opening with thiophenolate and successive MPM protection of the generated hydroxy group, followed by oxidation of thioether furnished compound **145**. A Pummerer rearrangement, subsequent hydrolysis and consecutive Luche propargylation delivered alkyne **146**. A homologation of alkyne **146** using formaldehyde and a secondary amine in presence of CuI furnished the allene **147** and **148**.

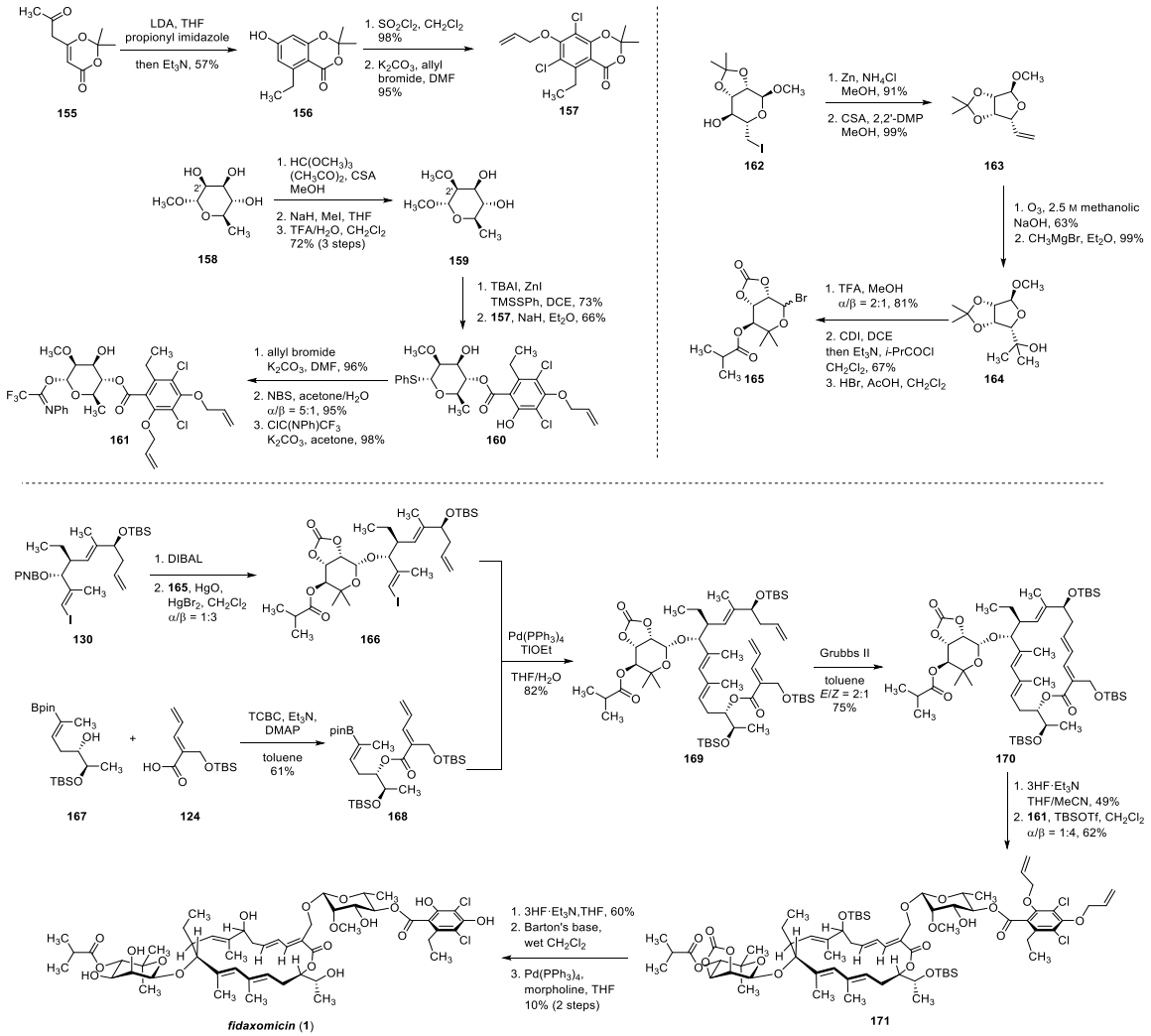
With those two fragments in hands, an alkyne/allene cross coupling was applied to connect the building blocks **143** and **148** to afford alkyne **149**. Installation of the methyl group was achieved *via* hydrosulfuration and application of a Kumada-Corriu coupling. Next, cross metathesis with alkene **153** and Suzuki cross coupling with bromide **154** delivered the aglycon scaffold **151**, which upon Yamaguchi macrolactonization gave the protected aglycon **152**.

Gademann's Total Synthesis of Fidaxomicin

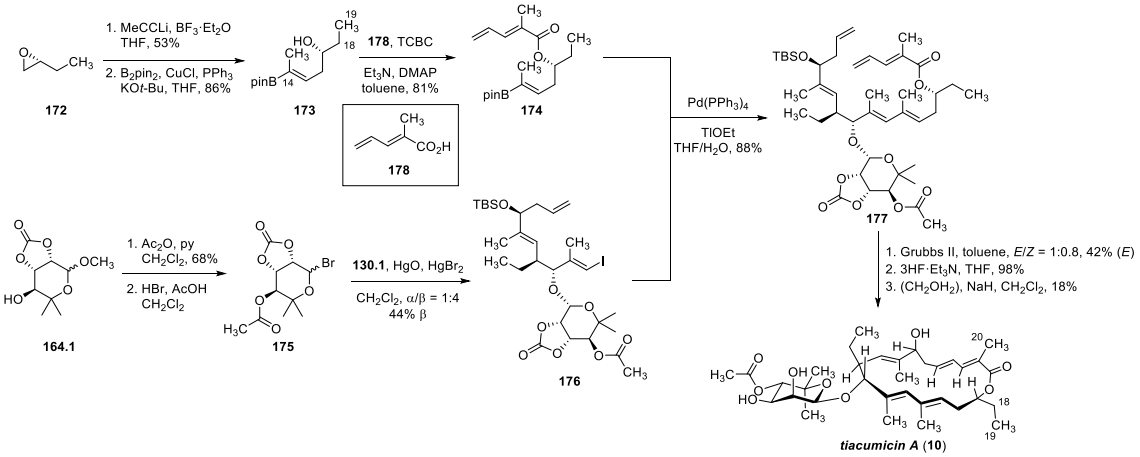
The first reported total synthesis of natural product **1** was achieved in the Gademann group in 2015.[66] After the successful synthesis of the core macrolactone **135**, the focus was set on the synthesis of the carbohydrate fragments **161** and **165** (*Scheme 7*). The dichlorohomoorsellinic acid moiety was constructed starting from dioxinone **155**. Claisen condensation and following cyclization/aromatization furnished phenol **156**. Next, dichlorination was achieved using sulfonyl chloride and subsequent allyl protection furnished the target building block **157**.

Starting from the known rhamnoside **158**, acetal protection of the *trans*-diol enabled selective methylation of C(2')-hydroxy group, followed by removal of the acetal under acidic aqueous conditions. Then, a thiophenyl group was installed in the anomeric position using ZnI and TMSSPh, which was then reacted with fragment **157** to furnish protected rhamnosyl-resorcylate **160**. The free phenolic hydroxy group was then allylated and a leaving group exchange in the anomeric position was required for optimal results at a later stage. Therefore, hydrolysis of thiophenol using NBS in aqueous acetone, followed by installation of trifluoroacetimidate gave glycosyl donor **161**. Next, the synthesis of the noviose moiety was commenced with iodopyranoside **162**. A Zn-mediated Vasella ring contraction followed by treatment with 2,2'-DMP and CSA in MeOH furnished furanose **163**. Ozonolysis in methanolic NaOH smoothly delivered the corresponding ester, followed by Grignard reaction with double addition of CH_3MgBr to form the *gem*-dimethyl moiety of **164**. Furanose **164** was then treated with TFA in MeOH to first achieve acetal deprotection and then induced ring expansion to obtain the desired pyranoside. Subsequently, the diol was protected with a carbonate group using CDI and the remaining hydroxy group was esterified with *i*-propylchloroformate. Finally, the anomeric position was brominated using HBr in AcOH to furnish the attempted glycosyl donor **165**. Having aglycon **135** already in hands, several attempts were made to perform the noviosylation.

Unfortunately, these attempts remained unsuccessful due to a low reactivity of the aglycon towards a variety of different glycosyl donors or exclusively unintended α-selectivity as described in a PhD thesis.[72]



Scheme 7. Total synthesis of fidaxomicin (**1**) by Gademann and coworkers. CSA: camphorsulfonic acid; TFA: trifluoroacetic acid; TBAI: tetrabutylammonium iodide; NBS: N-bromosuccinimide; 2,2'-DMP: 2,2'-dimethoxypropane; CDI: carbonyldiimidazole.



Scheme 8. Total synthesis of tiacumicin A (**10**) by Gademann and coworkers.

As a consequence, the synthetic strategy had to be reassessed and glycosylation with noviosyl bromide **165** was performed on macrocycle fragment **130** using Helferich's conditions before the aglycon was assembled. Next, Yamaguchi esterification of fragment **167** with carboxylic acid **124** delivered boronate **168**. Subsequently, a Suzuki cross coupling followed by ring closing metathesis yielded β-noviosylated aglycon **170**. After deprotection of the primary TBS group, rhamnosylation with acetimidate glycosyl donor **161** was performed and delivered
fully protected fidaxomicin **171** in good β-selectivity. Finally, global deprotection yielded the desired natural product **1** in moderate yields.

Gademann's Total Synthesis of Tiacumicin A

Tiacumicin A is a natural product that has been isolated along with antibiotic **1** from the fermentation broth. In comparison to **1**, tiacumicin A constitutes a mono-glycosylated variant of fidaxomicin aglycon, with lacking hydroxy groups in C(18)- and C(20)-position and an acetyl- instead of isobutyric ester on the noviose (*Scheme 8*). Based on the successful approach using early glycosylation on known fragment **130.1** as the key step, tiacumicin A was prepared.[67] The synthesis of the C(14)-C(19) fragment **173** with a lacking hydroxy group in C(18)-position started with epoxide opening of commercially available epoxide **172** with propynyl lithium in presence of $BF_3{\cdot}OEt_2$ and subsequent borylation of the generated alkyne to give boronate **173**. For the synthesis of the noviose glycosyl donor **175**, the common intermediate **164.1** from the total synthesis of **1** was used and the acetyl group was installed using acetic anhydride. Furthermore, replacement of the leaving group in the anomeric position was performed as previously described to furnish bromide **175**, which was carried on to glycosylation with known fragment **130.1**. Finally, the Suzuki cross coupling of iodide **176** with boronate **174** afforded intermediate **177** which was subsequently submitted to RCM in presence of Grubbs second generation catalyst. Upon removal of the protecting groups, the natural product **10** was obtained.

Norsikian and Roulland's Total Synthesis of Fidaxomicin

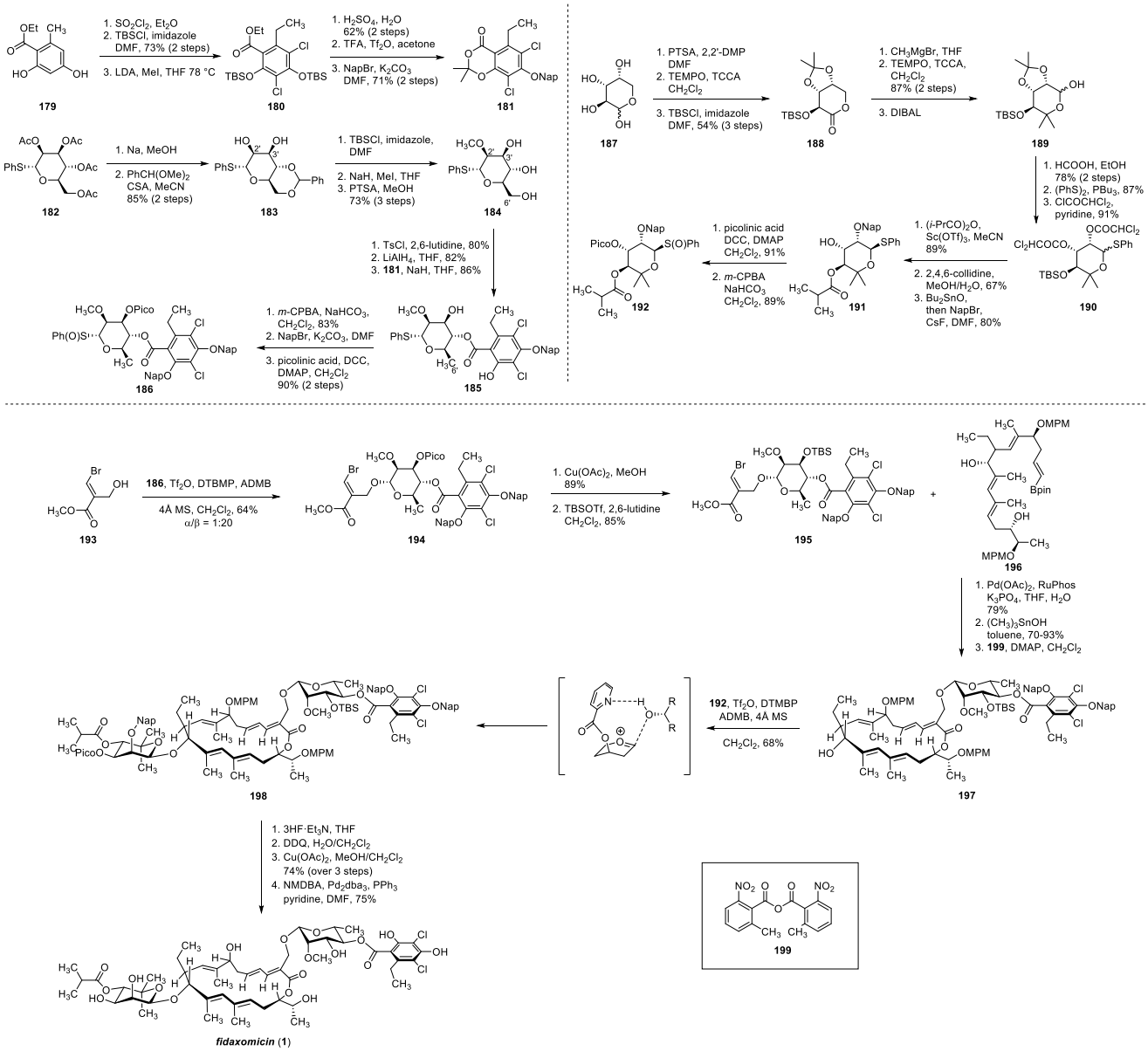
Recently, Roulland and coworkers published the second total synthesis of fidaxomicin, addressing the issue of the challenging β-selective glycosylations.[68]In contrast to the total synthesis of Gademann and coworkers, they performed the rhamnosylation (instead of the noviosylation) early on in the synthesis on a macrocycle precursor fragment (*Scheme 9*). The β-selective noviosylation was applied at a late stage on the fully installed macrocyclic part using a H-bond-directed β-glycosylation strategy.

The synthesis of the rhamnosyl-resorcylate moiety started from commercially available orsellinic acid analog **179**. Dichlorination with sulfuryl chloride, TBS-protection of the phenolic hydroxy group and benzylic methylation afforded the desired dichlorohomoorsellinic acid scaffold **180**. Next, acidic hydrolysis and subsequent acetonide protecting followed by re-protection of the free phenol delivered the fully protected glycosylation precursor **181**.

The synthesis of the rhamnosyl donor commenced with the deprotection of the known acetyl-protected mannopyranoside **182** using Zemplén's conditions, followed by benzylidene acetal protection of the obtained diol. Subsequently, selective TBS-protection of C(3')-OH, methylation at C(2')-OH, followed by global deprotection delivered triol **184**. Next, a tosyl protecting group was selectively installed at the primary hydroxy group, followed by reduction to install the desired C(6')-methyl group. Finally, glycosylation with homoorsellinate **181**, oxidation of the thiophenyl group with *m*-CPBA and installation of napthyl and picolinic acid protecting groups furnished the desired rhamnosyl-homoorsellinate glycosyl donor **186**. Furthermore, the noviosyl donor **192** was prepared starting from D-arabinose (**187**). Acetonide protection of the *cis*-vicinal diol, oxidation to the lactone and subsequent TBS-protection delivered lactone **188**. Grignard addition to the lactone resulted in the installation of *gem*-dimethyl group. The open diol was then re-oxidized using TEMPO and TCCA to obtain a lactone which was in turn reduced using DIBAL to furnish noviose **189**. Then, acetonide deprotection and conversion into thiophenyl glycosyl donor with subsequent protection of the free hydroxy groups gave **190**. In order to achieve high selectivity in the novioslyation of the macrocycle fragment, installation of suitable protecting groups was required. Therefore, several protection- and deprotection steps were carried out to obtain after oxidation of the anomeric thiophenyl group, the required glycosyl donor **192**.

Having, those fragments in hands, the assembly of the natural product **1** was attempted. After extensive screening, Roulland and coworkers found that glycosylation using rhamnosyl donor **186** and acceptor **192** *via* activation by Tf_2O in presence of DTBMP and ADMB delivered **194** in high β-selectivity (α/β = 1:20) due to an H-bond mediated Aglycon Delivery (HAD) mediated by the picoloyl protecting group. Next, a protecting group exchange from the picoloyl- to a TBS-protecting group was carried out. The assembly of the macrocyclic core **197** was achieved *via* a Suzuki cross coupling with known, advanced fragment **196**,[63] selective ester hydrolysis using Me_3SnOH and subsequent Shiina macrolactonization.

Next, the second glycosylation was performed with noviosyl donor **192** *via* HAD, which delivered the desired fidaxomicin scaffold **198** in high stereoselectivity (α/β > 1:20). After global deprotection, the second total synthesis of fidaxomicin (**1**) was successfully accomplished.



Scheme 9. Total synthesis of fidaxomicin (**1**) by Roulland and coworkers. Nap: napthyl, PTSA: *p*-toluenesulfonic acid; Ts: tosyl; *m*-CPBA: m-chloroperoxybenzoic acid; DCC: *N*,*N*′-dicyclohexylcarbodiimide; TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxyl; TCCA: trichloroisocyanuric acid; DTBMP: 2,6-di-tertbutyl-4-methylpyridine; ADMB: 4-allyl-1,2-dimethoxybenzene; DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; NDMBA: *N*,*N*′-dimethylbarbituric acid.

7. Conclusion

Among a variety of different naturally occurring fidaxomicin-like natural products, fidaxomicin possesses the highest activity against a broad panel of different bacterial strains. Although **1** was already discovered in the 1970s, it was not until 2011 when it was introduced to the market as an antibiotic for the treatment of CDI. Besides its antibacterial properties, also anti-cancer activities are reported for some fidaxomicin analogs. Regarding the current alarming situation concerning antibiotic resistance and the associated urgent need of new antibiotics, fidaxomicin analogs might be prospective antibiotic drug candidates. Recent insights into the biosynthetic pathway, mode of action and the experience gained through synthetic studies opens new perspective towards the design and synthesis of improved fidaxomicin derivatives.

Acknowledgements

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